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Chemical Characterization of Lipid A from Some Marine Proteobacteria

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Abstract—Lipids A from type and wild strains of marine Proteobacteria belonging to *Alteromonadaceae* (*Alteromonas* (1 species), *Idiomarina* (1 species), and *Pseudoalteromonas* (8 species) genera) and *Vibrionaceae* (*Shewanella* (1 species) and *Vibrio* (1 species) genera) families and *Marinomonas* genus (1 species) were isolated by hydrolysis of their respective lipopolysaccharides with 1% acetic acid. Based on thin-layer chromatography data, the lipids A studied had low heterogeneity and generated family-specific patterns varying in numbers of bands and their chromatographic mobility. Total chemical analysis of the compounds showed that they contained glucosamine, phosphate, and fatty acids with decanoate (*I. zobellii* KMM 231^T lipid A) or dodecanoate (lipids A of the other bacteria) and 3-hydroxy alkanoates as the major fatty acid components. Unlike terrestrial bacterial lipids A, lipids A of marine Proteobacteria had basically monophosphoryl (except *V. fluvialis* AQ 0002B lipid A with its two phosphate groups) and pentaacyl (except *S. alga* 48055 and *V. fluvialis* AQ 0002B lipids A which were found to have six residues of fatty acids per molecule of glucosamine disaccharide) structural types, low toxicity, and may be useful as potential endotoxin antagonists.

Key words: marine Gram-negatives, lipid A, thin-layer chromatography, fatty acid, toxicity, endotoxin antagonist

Gram-negative bacteria, along with classical membrane lipids based on glycerol, contain an unusual glycophospholipid known as lipid A. Performing the function of a lipid anchor for lipopolysaccharide (LPS), one of the main components of the bacterial outer membrane [1], it is a component of the cell wall and is important for maintenance of normal physiology and growth of the microorganisms [2]. LPS, O-antigens, and endotoxins of Gram-negative bacteria constitute a specific class of biopolymers that have a wide spectrum of biological action [3]. Many pathophysiological manifestations of gram-negative infections, including endotoxemia and bacterial shock, are associated with unique, so-called endotoxic properties of LPS [4].

Modern medicine has excellent antibacterial means against Gram-negatives as such, but effective therapy against endotoxemia and septic shock is lacking. There is compelling evidence that inherent endotoxic activities of LPS are expressed by a lipid fragment of its molecule, lipid A [3-5]. Therefore, intensive searches for potential endotoxin antagonists on the basis of lipid A, including those which interrupt the synthesis of lipid A, bind or neutralize its action, prevent interactions of lipid A with host effector cells, and interfere with lipid-mediated signal transduction pathways, are now being carried out [6].

A structure common to a number of lipids A is made by a β-1,6-D-glucosaminyl-D-glucosamine [7]. The disaccharide molecule has α-glycosidic (position 1) and non-glycosidic (position 4′) phosphoric groups and is acylated with amide-linked (at positions 2 and 2′) and ester-linked (at positions 3 and 3′) R-3-hydroxy- and R-3-acyloxy fatty acids. Based on the number of fatty acid residues in a molecule of lipid A, penta-, hexa-, and heptaacyl structural types are recognized [8]. All endotoxically active lipid A molecules have the hexaacyl type of structure and two residues of phosphoric acid [9]. On the other hand, according to data available so far, lipid A structural variants displaying high endotoxin antagonism have a disaccharide backbone, two or one phosphate groups, and low acylation degree [10-13]. Lipid A acylation patterns which play an important role in the binding of bacteria with and the activation of host cells [3, 5] are

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known strongly to depend on growth conditions [14-16]. We hypothesized that marine bacteria whose habitat is characterized by a specific environment, such as a generally low temperature, high hydrostatic pressure, and increased salt concentration [17], may provide lipids A of pharmacological interest.

To date, lipid A studies have been mostly focused on terrestrial organisms [7, 8]. Although an examination of the lipids A from some marine bacteria has been reported, their characterization included only the identification of fatty acids [18, 19]. This report describes total compositional analysis, thin-layer chromatographic behavior, and toxic properties of the lipids A from several marine Proteobacteria. Type and environmental strains of *Alteromonas*, *Idiomarina*, and *Pseudoalteromonas* (family *Alteromonadaceae*) [20], *Shewanella* and *Vibrio* (family *Vibrionaceae*) [21], and *Marinomonas* genera, which are common habitants of marine environments and comprise a diverse group of true marine bacteria, were chosen as the subject matter of the present investigation.

MATERIALS AND METHODS

Strains and growth conditions. The studied strains of marine bacteria, with information on source of isolation, are listed in Table 1. A total of 13 bacterial cultures were used, 11 of which were marine, i.e., were isolated from open ocean water or marine sediments, and 2 (*Shewanella alga* 48055 and *Vibrio fluvialis* AQ 0002B) were clinical samples isolated from patients with infectious illness whose causative agents were marine bacteria. For comparative purpose, one terrestrial bacterial strain, *Yersinia pseudotuberculosis* O1:b, was also included in this study.

With the exception of *Y. pseudotuberculosis* O1:b, whose cultivation conditions were described in [16], all strains were routinely grown aerobically at room temperature in a medium containing marine water [22]. Cells in the late logarithmic phase of growth were centrifuged at 5000*g*, washed consecutively in equal volumes of saline and acetone, and air dried.

Isolation of LPS and lipid A. Lipopolysaccharides obtained by extraction of dry bacterial cells with hot aqueous phenol were recovered from the dialyzed aqueous–phenolic phase as described in previous studies [23-25]. To obtain free lipids A, LPS (2 to 3 mg) were hydrolyzed with 1% acetic acid (1 ml) at 100°C for 1.5 to 2 h (with exception of *P. distincta* KMM 638^T lipid A, the isolation of which required 40 min). The precipitated fractions, corresponding to lipids A, were separated by centrifugation. The purified by washing with water followed by drying with anhydrous sodium sulfate and precipitating with acetone lipid A preparations were stored in chloroform or chloroform–methanol (2 : 1) solutions (1-2 mg/ml) in the cold.

Thin-layer chromatography (TLC) of the lipid A preparations. Chromatograms were run on ready-made aluminum-backed Sorbfil plates of silica gel (Sorbpolymer). Lipid A samples were studied using the following solvent systems: chloroform–methanol–H_2O–concentrated aqueous ammonia (10 : 6.25 : 1 : 0.5 v/v; lipid A); hexane–diethyl ether–AcOH (7 : 3 : 0.1 v/v; free fatty acids and methyl esters of fatty acids). Bands were visualized by heating the plates at 130°C for 10 min after spraying with 10% H_2SO_4 in methanol.

Fatty acid analysis. Fatty acids (FA) were prepared by hydrolysis of lipids A (1 mg) with 6 M NaOH at 100°C for 4 h. Samples were allowed to cool, acidified with 6 M HCl, and free FAs were extracted twice with chloroform. Chloroform extracts were washed with water and dried with anhydrous sodium sulfate. Methyl esters of fatty acids (FAMEs) were obtained by treatment of dry

Table 1. List of strains studied

No.	Strain	Other designations	Source
1	*Alteromonas macleodii* ATCC 27126^T	IAM 12920^T, NCIMB 1963^T	ATCC
2	*Idiomarina zobellii* KMM 231^T		KMM
3	*Marinomonas vaga* ATCC 27119^T		ATCC
4	*Pseudoalteromonas atlantica* IAM 12927^T	NCIMB 301^T, ATCC 19262^T	IAM
5	*Pseudoalteromonas distincta* KMM 638^T	ATCC 700518^T	KMM
6	*Pseudoalteromonas elyakovii* KMM 162^T	ATCC 700519^T	KMM
7	*Pseudoalteromonas espejiana* ATCC 29659^T		ATCC
8	*Pseudoalteromonas haloplanktis* IAM 127915^T	ATCC 14393^T, NCIMB 2084^T	IAM
9	*Pseudoalteromonas nigrifaciens* IAM 13010^T	ATCC 19375^T, NCIMB 8614^T	IAM
10	*Pseudoalteromonas tetraodonis* IAM 14160^T		U. Simidu
11	*Pseudoalteromonas undina* ATCC 29600^T		IAM
12	*Vibrio fluvialis* AQ 0002B		S. Shinoda
13	*Shewanella alga* 48055		B. F. Vogel

residues with diazomethane in ether. Calculated amounts of pentadecanoic acid were added to the lipids A before hydrolysis as an internal standard.

FAMEs were identified by gas–liquid chromatography (GLC) and GLC–mass spectrometry (GLC-MS). GLC analysis was performed with a GC-9A chromatograph (Shimadzu, Japan) with Supelcowax 10 and SPB-5 columns (both of size 30 m × 0.25 mm) at temperature of 200 and 210°C, respectively [22]. GLC-MS analysis was carried out on a Hewlett-Packard model 6890 gas chromatograph equipped with capillary column HP 5 MS 5% Phenyl Methyl Siloxane (30 m × 250 μm × 0.25 μm) connected to a Hewlett-Packard model 5973 mass spectrometer. Samples were injected in the split mode with a split ratio of 1 : 15 at injector temperature 250°C. The oven was temperature programmed to increase from 150 to 210°C at rate of 5°C/min. Helium was used as the carrier gas. MS operating conditions were as follows: electron multiplier, 1800 V; transfer line, 250°C; electron impact energy, 70 eV; spectra were registered in a mass range of 50 to 550 atomic mass units with 2.94 scans per second.

Carbohydrate analysis. Free amino compounds were qualitatively and quantitatively analyzed using an LKB 4251 Alpha Plus system after complete acid hydrolysis (4 M HCl, 100°C, 24 h) of lipid A preparations. In the case of alditol acetates, the samples were hydrolyzed (4 M HCl, 100°C, 24 h), reduced, and acetylated. Then GLC was carried out on a Pye Unicam-104 gas–liquid chromatograph fitted with glass columns (2.5 m × 3 mm) packed with 3% QF-1. For GLC-MS of polyol acetates, an LKB-9000S instrument was used; it was equipped with columns packed with 5% SE-30.

Phosphate analysis. Total phosphorus generated after charring of the lipids A with $HClO_4$ was determined by the ammonium molybdate method as described in [26].

Lethal toxicity. The toxic properties of the lipids A were tested in outbred D-galactosamine-sensitized mice (16-18 g) by the method of Galanos [27]. D-Galactosamine-hydrochloric acid (16 mg per animal) and different amounts of lipids A (0.004 to 4 mg) were administered as mixtures in 0.4 ml of phosphate-buffered saline intraperitoneally into groups of four animals. A control of 4 mice was injected with saline only. Mortality was monitored for 48 h. Toxicity was expressed as LD_{50} and was calculated by the Nowotny method [28].

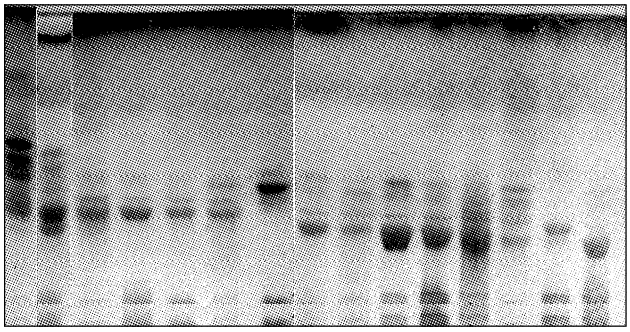
Statistical analysis. All the data presented here are means of three or more independent experiments. The range of experimental errors was less than 5%.

RESULTS

TLC of lipids A. Lipids A from marine bacteria (marine lipids A) obtained by hydrolysis of the corresponding LPS with 1% acetic acid [23-25] were examined by TLC (figure). A complex pattern of components, similar to that of the enteric *Y. pseudotuberculosis* O:1b lipid A (figure, lane *1*, see also [29]), was detected only for the *S. alga* 48055 and somewhat the *V. fluvialis* AQ 0002B lipids A (figure, lanes *13* and *2*, respectively). Other marine lipids A exhibited simpler TLC profiles of four bands each, with a significant prevalence of one of them.

The *M. vaga* ATCC 27126^T lipid A was of high chromatographic mobility (R_f = 0.65; figure, lane *7*). Other lipids A were less mobile, displayed surprisingly uniformly TLC sets, and contained one major band (R_f = 0.37) and several bands with lesser intensity (figure, lanes *4-6*, *8-12*, *15*). The only exception was the *P. atlantica* IAM 12376^T lipid A, which had the same complement of bands in TLC (figure, lane *14*), but its major compound was of greater chromatographic mobility (R_f = 0.42) than the main component of other *Pseudoalteromonas* lipids A.

Four various TLC patterns, with amount of bands and their chromatographic mobility as a criterion, are seen (figure). Type 1 consists of the *M. vaga* ATCC 27119^T lipid A. Type 2 lipid A was detected in *A. macleodii* ATCC 27126^T, *I. zobellii* KMM 231 and practically in all *Pseudoalteromonas* strains studied (except the *P. atlantica* IAM 12376^T lipid A, whose untypical TLC profile remains unclear). Type 3 includes the *S. alga* 48055 and *V. fluvialis* AQ 0002B lipids A that were the most heterogeneous among the marine lipids A studied. Maximal heterogeneity was observed in TLC pattern of lipid A from *Y. pseudotuberculosis* O:1b serovar.



Thin-layer chromatography of lipid A preparations. Samples of lipid A were fractionated on Sorbfil plates (Sorbpolymer) using chloroform–methanol–concentrated NH_4OH–water (10 : 6.25 : 1 : 0.5 v/v). Bands were detected by spraying the chromatograms with 10% H_2SO_4 in methanol followed by heating at 120°C. Samples of lipid A are presented as follows: *1*) *Y. pseudotuberculosis* O:1b serovar; *2*) *V. fluvialis* AQ 0002B; *3*) *P. haloplanktis* IAM 12915^T; *4*) *P. tetraodonis* IAM 14160^T; *5*) *P. elyakovii* KMM 162^T; *6*, *9*) *I. zobellii* KMM 231^T; *7*) *M. vaga* ATCC 27119^T; *8*) *P. distincta* KMM^T; *10*) *P. espejiana* ATCC 29659^T; *11*) *P. undina* IAM 12922^T; *12*) *P. nigrifaciens* IAM 13010^T; *13*) *S. alga* 48055; *14*) *P. atlantica* IAM 12376^T; *15*) *A. macleodii* ATCC 27126^T.

Table 2. Fatty acid composition of lipids A from some marine Proteobacteria

Fatty acid	Content of fatty acids, % of total fatty acids												
	A. macleodii	*I. zobellii*	*M. vaga*	*P. atlantica*	*P. distincta*	*P. elaykovii*	*P. espejiana*	*P. haloplanktis*	*P. nigrifaciens*	*P. tetraodonis*	*P. undina*	*S. alga*	*V. fluvialis*
	1	2	3	4	5	6	7	8	9	10	11	12	13
10 : 0		10.8		1.3			traces		1.0	1.1	1.6		
11 : 0					2.9		0.8	1.7	0.6	1.4	2.0	0.6	
12 : 0	17.1	2.0	9.3	12.4	5.9	13.7	10.3	10.7	12.8	15.9	11.1	11.2	10.8
13 : 0	0.5				2.3		0.7	1.9	1.8	0.5	1.4	3.0	
i13 : 0		6.5		13.5	0.8			0.8				13.2	2.3
14 : 0	2.6				0.5	8.0	traces	0.6	1.2	1.2	1.7	1.1	16.0
15 : 0	1.5	1.1				0.8	traces		0.7	0.6	1.1	0.5	1.7
16 : 0	8.6	3.1	2.4		2.7	5.0	2.4	2.8	7.7	5.3	6.7	2.9	10.2
18 : 0	3.8		0.6			2.4	0.9	0.9	1.7	2.7	2.1	1.0	2.9
12 : 1					1.9		1.6					0.6	
14 : 1							0.8	0.8		0.8			
16 : 1ω7	2.2		0.9			1.3			1.7	1.7	1.7	1.0	3.5
18 : 1ω9	2.9					1.1	0.85	0.6	1.5	0.6	1.9	0.6	2.9
18 : 1ω7						2.1			0.4	0.9		0.5	
2OH-12 : 0						4.8							
3OH-10 : 0	11.9	9.0	75.0		6.0	8.2	14.5	4.6	2.6	7.8	5.2	0.9	
3OH-11 : 0	4.2			3.1	26.7		9.4	17.6	7.9	5.3	11.5	3.7	1.0
3OH-12 : 0	9.7	7.2	1.5	23.4	22.9	15.5	39.0	32.5	32.7	31.0	32.4	16.6	14.7
3OH-13 : 0	1.6			4.0	8.3	0.6	3.5	7.3	5.0	2.1	5.2	7.2	1.7
3OH-14 : 0	25.0			15.9		25.0	0.9	0.7	6.2	1.0	0.9	7.5	20.2
3OH-16 : 0													1.8
i3OH-11 : 0		28.1			5.0		2.9	4.6		2.7	4.9	0.7	
i3OH-12 : 0					3.8		1.4	3.0	3.4	5.7	1.8		
i3OH-13 : 0		31.4		15.7			0.6	1.0		0.8	1.8	12.8	
i3OH-14 : 0													2.4
3OMe-12 : 0							1.5		1.3	1.0	0.7		
3OMe-14 : 0					0.6	1.1							
$\Delta^2$10 : 1			3.6										
$\Delta^2$11 : 1								1.0					
$\Delta^2$12 : 1				3.5	3.8	1.3	0.6	2.2	0.7	0.5		2.6	6.0
$\Delta^2$13 : 1		1.8		1.7				0.8					
$\Delta^2$14 : 1	3.5			2.4		6.0			2.5			0.7	1.8
$\sum\Delta^2 n$: 1	3.5	1.8	3.6	7.6	3.8	6.3	0.6	4.0	3.2	0.5		3.3	7.8
∑3OH*	55.9	77.5	80.1	69.7	77.1	62.5	74.3	75.3	62.3	57.9	65.1	52.7	49.6

* In this row, the 3-hydroxy acid contents involve the content of Δ^2-unsaturated fatty acids formed from acyloxy esters under alkaline hydrolysis conditions [31], 2-hydroxy-, and 3-methoxy fatty acids.

It is of interest that the organisms studied (*Y. pseudotuberculosis* included) belong to three various families—*Enterobacteriaceae* (*Y. pseudotuberculosis* O:1b [21]), *Vibrionaceae* (*S. alga* 48055 and *V. fluvialis* AQ 0002B [21]), and a newly created family *Alteromonadaceae* (*A. macleodii* ATCC 27126^T, *I. zobellii* KMM 231^T, and 9 species of *Pseudoalteromonas* genus [20])—and *Marinomonas* genus (*M. vaga* ATCC 27119^T) that is in line with the number of the TLC profiles observed. A correlation between the lipid A TLC patterns on one hand and taxonomical position of bacteria from which lipid A was isolated on the other hand has obviously been drawn. However, additional studies are necessary to confirm it.

Fatty acid compositions. The composition of FAs obtained by drastic alkaline hydrolysis of lipids A are given in Table 2. The acids listed were identified by the retention times of their methyl esters using standard fatty acids and examination of each fraction by GLC-MS. The spectra of normal fatty acid methyl esters contained fairly abundant M^+ ions. The mass-spectra of 2-hydroxy-, 3-hydroxy-, 3-methoxy- and *trans*-Δ^2-unsaturated FAs contained the expected base peaks at *m/z* 199 (2-hydroxy fatty acids), *m/z* 103 (3-hydroxy fatty acids), *m/z* 117 (3-methoxygroups), *m/z* 87 (*trans*-Δ^2-unsaturated fatty acids) and other diagnostic peaks of lesser intensity characteristic for each type of acids [30].

Lipid A of *M. vaga* ATCC 27119^T contained one type of 3-hydroxy acids that was identified as 3OH10:0 and was shown to be prevalent (75% of the total FA content, Table 2). The 3-hydroxy acid profiles of other lipids A were more complex and consisted of several fatty acids of this type. Major 3-hydroxy acids were 3OH11:0 (*P. distincta* KMM^T lipid A), 3OH12:0 (detected in lipid A of twelve from thirteen species of marine bacteria studied), 3OH14:0 (*A. macleodii* ATCC 27126^T, *P. elyakovii* KMM 162^T, and *V. fluvialis* AQ 0002B lipids A). The *S. alga* 48055, *P. atlantica* IAM 12376^T, and *I. zobellii* KMM 231 lipids A contained also branched-chain 3-hydroxy fatty acids of the *iso*-series in significant amounts (from 12 to 60%, Table 2).

The ranges of non-hydroxy fatty acids were shown to be similar in most of the lipids A studied and 12:0 was the major acid of this type in many samples. Exceptions were *M. vaga* ATCC 27119^T lipid A (along with 12:0, it contained 10:0, Table 2), *P. atlantica* IAM 12376^T, *S. alga* 48055, and *V. fluvialis* AQ 0002B lipids A (in addition to 12:0, they contained i-13:0 or 14:0; Table 2, columns 4, 12, 13, respectively) and the *I. zobellii* KMM 231 lipid A, which contained 10:0 and i-13:0 instead of 12:0 (Table 2, column 2).

Trans-Δ^2-unsaturated acids, characteristic for many enteric lipid A hydrolyzates [31] and indicative of the presence of acyloxy esters in the lipid A preparations (Δ^2-*trans*-unsaturated derivatives are preferably formed from ester-linked 3-acyloxyacyl residues [31]) occurred in small and even trace amounts (Table 2). At the same time, rather high amounts of 16:0, 16:1, 18:0, 18:1 acids were observed in some samples. Based on ESI-MS data (unpublished results), they do not belong to lipid A fatty acids and seem to be components of other membrane lipids, such as phospholipids or lipoproteins that form often difficult-to-separate complexes with LPS.

Identification of lipid A backbone. Sugar analysis of the lipids A carried out by acid hydrolysis followed by reduction, O-acetylation, and GLC-MS procedures, on one hand, and analysis of underivatized amino compounds by amino acid analyzer, on the other hand, showed the presence of glucosamine as the only monosaccharide. After charring with $HClO_4$, all investigated compounds gave colored complexes with phosphomolybdate that indicates the presence of phosphoric acid residues and suggests that they are phospholipids.

Total chemical analysis of lipid A. Using methods of quantitative analysis, amounts of glucosamine, phosphorus, and main fatty acids in some lipids A were measured (Table 3). On a molar basis, it is possible to assume that one (or two for *V. fluvialis* AQ 0002B lipid A) phosphate group and a total of six (*S. alga* 48055 and *V. fluvialis* AQ 0002B lipids A) or five (lipids A of other bacteria) fatty acyl chains per 2 mol of glucosamine were present in the studied preparations.

Lethal toxicity. The toxic properties of several marine lipids A (in comparison with that of the *Y. pseudotuberculosis* O:1b serovar LPS) are given in Table 3. It appears that the marine lipids A studied had significantly higher lethal doses (LD_{50} for *P. espejiana* ATCC 29659^T, *I. zobellii* KMM 231, *M. vaga* ATCC 27119^T, *S. alga* 48055, and *V. fluvialis* AQ 0002B lipids A were 4.85, 3.12, 2.00, 1.46, and 0.17 mg per animal) than *Y. pseudotuberculosis* O:1b serovar LPS LD_{50}, which was 0.063 mg per animal.

DISCUSSION

The studies presented here were designed to determine the marine lipid A structural types, their distribution between bacteria of various genera and families, specific features conditioned by marine habitat, toxic activities of some of them, and, on the basis of the data obtained, to evaluate the possibilities of using marine lipids A as potential endotoxin antagonists.

In general, the marine lipids A showed many of the characteristics of terrestrial lipid A molecules: they contained glucosamine, phosphate, and fatty acids with dodecanoate and 3-hydroxy alkanoates as main fatty acid components. At the same time, the study revealed some exceptional properties in the marine lipids A, the most pronounced among which were the presence only of one phosphate group and basically pentaacyl type of structure. The *I. zobellii* KMM 231 lipid A is a special case because of lack of dodecanoic acid, a fatty acid which is

Table 3. Chemical composition of lipids A from some marine Proteobacteria

Component	Content of the components of lipid A from different sources															
	I. zobellii KMM 231^T			*M. vaga* ATCC 27119^T			*P. espejiana* ATCC 29659^T			*S. alga* 48055			*V. fluvialis* AQ 0002B			*Y. ps-s*
	%	µmol/mg	mole*	%	µmol/mg	mole*	%	µmol/mg	mole*	%	µmol/mg	mole*	%	µmol/mg	mole*	LPS
10 : 0	5.05	0.0326	0.50	3.63	0.0234	0.40	1.12	0.0072	0.10							
12 : 0	0.95	0.0052	0.09	4.87	0.0266	0.45	7.20	0.0393	0.57	10.45	0.0571	0.97	5.40	0.0295	0.64	
i13 : 0	3.04	0.0154	0.26				1.08	0.0049	0.07	12.77	0.0648	1.11	1.16	0.0059	0.12	
14 : 0										1.04	0.0050	0.08	8.01	0.0380	0.82	
16 : 0	1.46	0.0061	0.05	1.26	0.0053	0.09	1.93	0.0081	0.23	2.66	0.0110	0.19	5.13	0.0215	0.46	
3OH-10 : 0	5.67	0.0332	0.50	41.09	0.2415	4.12**	10.18	0.0595	0.86							
3OH-11 : 0							6.59	0.0356	0.52	3.5	0.0186	0.32	1.08	0.0058	0.13	
3OH-12 : 0	4.52	0.0227	0.38	0.78	0.0039	0.07	27.76	0.1459	2.02**	17.95	0.0914	1.56**	19.41	0.1014	2.19**	
3OH-13 : 0							2.39	0.0112	0.16	6.70	0.0315	0.54	1.67	0.0078	0.17	
3OH-14 : 0							0.61	0.0026	0.04	7.8	0.0341	0.61**	10.91	0.0485	1.05**	
3OH-16 : 0													1.80	0.0075	0.16	
i3OH-11 : 0	17.72	0.0958	1.60				2.01	0.0109	0.16							
i3OH-12 : 0							0.96	0.0048	0.07							
i3OH-13 : 0	21.75	0.1025	1.71**				0.43	0.0020	0.03	11.96	0.0561	0.96				
i3OH-14 : 0													4.37	0.0192	0.41	
3OMe-12 : 0								0.0049	0.07							
Σ_{norm}FA			0.90			0.94			0.97			2.11			2.04	
Σ3OH			4.24			4.12		1.05	3.93			3.99			4.11	
P	2.3	0.0752	1.25	2.13	0.0687	1.17		0.0764	1.10	1.75	0.0564	0.96	2.69	0.0868	1.88	
GlcN	20.34	0.1197	2.00	19.91	0.1171	2.00		0.1387	2.00	19.92	0.1172	2.00	15.72	0.0925	2.00	
LD_{50}***			3.12			2.0		2.37	4.85			1.46			0.166	0.063
								23.59								

* In this row, moles of components per two moles of glucosamine were calculated.
** Amounts of these 3-hydroxy acids were expressed as sums of 3-hydroxy- and corresponding Δ^2-unsaturated acids [31].
*** LD_{50} were determined by the Galanos method [27] and calculated by the Nowotny method [28].

commonly present in most of the wild-type terrestrial lipids A ([32], see also Table 2), and the predominance of odd-numbered 3-hydroxy fatty acids of iso-series (87% of total amount of this type FAs).

Another feature of marine lipids A is the small quantity or even complete absence of *trans*-Δ^2-unsaturated FAs in the composition of their molecules. This seems likely to be determined by underacylaion of marine lipids A (in comparison with lipids A of terrestrial microorganisms, especially with lipid A of enteric bacteria, which have basically hexaacyl type of substitution [2, 8]). A low content of *trans*-Δ^2-unsaturated FAs gives an indication of absence of ester-linked acyloxy FAs in marine lipids A [31] and is in good agreement with data that the majority from them contains only five FA residues.

A low acylation degree in marine lipids A appears to arise from conditions of their habitat. Most of them live in open ocean with its relatively low temperature and by virtue of this display properties of psychrotolerant microorganisms, which, as was previously shown on other bacteria [29, 33], synthesize acyl-deficient partial lipid A structures at room temperature. It is interesting that the most similarity with lipid A from terrestrial bacteria of the enteric group was displayed by lipids A from *S. alga* 48055 and *V. fluvialis* AQ 0002B, which, unlike other marine bacteria isolated from the open ocean waters and marine sediments, are clinical isolates and synthesize molecules of lipids A with hexaacyl type structure.

One more notable feature of the lipids A studied is a low degree of their heterogeneity. Heterogeneity of lipid A found to be a consequence of variation of the number and composition of carboxylic acid residues and varying phosphate content is an intrinsic characteristic of lipids A from many terrestrial bacteria. Based on TLC [34, 35] (see also figure, lane *1*) and mass-spectrometry [36] data, from 8 up to 12 individual components of various composition can be present in lipid A preparations. TLC profiles of marine lipids A containing four bands with significant prevalence of one of them seem unusual and suggest that cells of most of the bacteria investigated by us synthesize basically one type of lipid A molecules. Low heterogeneity of marine lipids A may derive from the monophosphoryl nature of most of them (Table 3), this reducing twofold the number of possible structural variants.

In general, any deviation (in increasing or decreasing directions) of the number of acyl residues present in lipid A from optima of six reduces endotoxic activity of lipid A and LPS [3, 5]. Consistent with this observation, underacylated marine lipid A were of lower lethal toxicity than lipids A of clinical isolates having six acyl residues (*S. alga* 48055 lipid A) and especially six acyl residues and two phosphate groups (*V. fluvialis* AQ 0002B lipid A) and displaying the most activity in this test (Table 3). Structural types of lipid A harboring a smaller number of fatty acids including pentaacyl lipid A are of interest as endotoxin antagonists [10-13]. Among the lipid A partial structures that can potentially protect against Gram-negative septic shock, monophosphoryl lipid A is also well known [37]. The fact that the major species of the marine lipid A structures is a pentaacyl monophosphoryl derivative of glucosaminobiose suggests that lipids A of marine bacteria may become a promising source of new medicinal preparations, potential endotoxin antagonists.

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